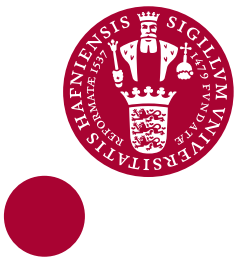

Relaxin boost may be facilitated by formation of intracellular vacuoles in luteal cell of the porcine ovary

an immunocytochemistry and transmission electron microscopy study

Dantzer, Vibeke; Fjälman, Kerstin

Published in:
Placenta

Publication date:
2010

Document version
Publisher's PDF, also known as Version of record

[P1.48]

RELAXIN BOOST MAY BE FACILITATED BY FORMATION OF INTRACELLULAR VACUOLES IN LUTEAL CELL OF THE PORCINE OVARY: AN IMMUNOCYTOCHEMISTRY AND TRANSMISSION ELECTRON MICROSCOPY STUDY

Vibeke Dantzer*, Kerstin Fjällman, LIFE, Copenhagen University, Denmark

Introduction: Relaxin is best known as a reproductive peptide hormone essential during late pregnancy as it promotes growth and softening of the uterine cervix, thereby ensuring rapid and safe delivery and secondly it also promotes growth and development of the mammary apparatus. New physiological functions for this hormone in implantation, vascularization and placentation is emerging, but with great diversity in the physiology and biological effect among species. In the pig the the corpus luteae synthesize this hormone early in gestation with a progressive increase from day 20 to reach a peak at late gestation and then a markedly decline at the onset of parturition. The pattern of accumulation in luteal cells ensuring the boost was the topic for this investigation.
Methods: Corpus luteum (2-3 per stage) from13 stages were investigated, 5 stages (day 33-95) were fixed in glutaraldehyde and processed for electron microscopy (TEM) and 8 (day 55-90 and one nonpregnant in early luteal phase) were fixed in Bouins fixative for immunocytohemistry using a polyclonal relaxin antibody (Immunediagnostic) both by rutine laboratory methods. All controls were negative.
Results and discussion: In the nonpregnant a very weak reaction was shown, and no apparent vacuoles were seen. Cellular vacuoles increased in number per field to day 95. The Relaxin reaction often surrounded these vacuoles and in 10μm sections reaction products were also seen in the lumen of the vacuoles. By TEM the vacuoles varied in size from 1,2μm to 25μm and shape – round, oval or irregular and electron dense granules and their products seen in the lumen. A basal lamina surrounded all lutein cells, but were never seen lining the vacuoles. The lutein cell wall arround parts of the vacuoles were very thin, 0.1μm, often seen close to capillaries.
Conclusion: The vacuoles are storing relaxin facilitating fast and important boost prior to parturition.
Keywords: relaxin, ovary, preparturition effect

[P1.49]

LEPTIN ENHANCES CELL PROLIFERATION AND SURVIVAL IN PLACENTAL CELLS

F Ibarbalz, J Maymó, Y Gambino, B Maskin, JC Calvo, C Varone*, [1]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina, [2]Hospital Alejandro Posadas, Argentina

Fetal-maternal dialogue during implantation involves multiple regulators such as leptin. This 16KD protein plays diverse roles in placental growth and survival.
Previous results from our group demonstrated that leptin increases cell proliferation and survival in JEG-3 and BeWo cells. We also demonstrated that leptin expression is tightly regulated by different placental regulators. The aim of the present work is to study the mechanisms involved in placental proliferation and apoptosis.
Methods: BeWo and Swan cells, and human term placental explants were used. Western blot analyses were carried out to detect leptin, Bcl-2, Bax and p53 expression. Cell proliferation was determined by cell counting and 3H-thymidine incorporation. Transfection assays with reporter constructs were used to determine leptin effect on different transduction pathways.
Results: Leptin treatment in Swan cells increased cell proliferation up to 3 times. Maximal effect was achieved with 100 ng leptin/ml at two days of incubation. Caspase-3 activation was determined by Western blot. Leptin diminished the proteolysis of caspase-3 in a dose dependent manner. Moreover the diminution in endogenous leptin by treatment with an antisense oligonucleotide (2-4 μM) increases cellular apoptosis measured by caspase-3 activation. Bcl-2 and Bax levels were determined after leptin treatment and the relationship between them calculated. The expression of the key cell cycle regulator p53 was also determined. Slightly changes were observed
Conclusions: All these results reinforce the notion of leptin as a placental cytokine with the function of promoting growth and survival of placental cells.
Keywords: leptin, placenta, proliferation, apoptosis